Serial No.: 10/601,319 Filed: June 20, 2003

Page : 6 of 21

D1370-14US PATENT

Amendment to the Claims:

Please amend the claims as follows:

Please cancel claims 13 to 17, without prejudice or disclaimer.

This listing of claims will replace all prior versions, and listing, of claims in the application:

Listing of Claims:

Claim 1 (currently amended): A method for making a polypeptide having a phytase activity comprising:

providing a nucleic acid <u>isolated</u> derived from <u>an E. coli</u> [[a]] bacteria encoding a polypeptide having a phytase activity; and

expressing the nucleic acid in a yeast under conditions which allow expression of the enzyme in the yeast.

Claim 2 (currently amended): The [[A]] method of claim 1, for making a polypeptide having a phytase activity comprising:

providing wherein the nucleic acid expressed in the yeast comprises a non-natural or synthetically generated nucleic acid-encoding-a-polypeptide having a phytase activity;

expressing the nucleic acid in a yeast under conditions which allow expression of the enzyme in the yeast.

Claim 3 (currently amended): The method of claim 1-or-claim 2, wherein the nucleic acid isolated from an *E. coli* bacteria has the [[a]] sequence of as set forth in SEQ ID NO:1, or wherein the polypeptide encoded by the nucleic acid isolated from an *E. coli* bacteria has the [[an]] amino acid sequence of as set forth in SEQ ID NO:2.

Claims 4 and 5 (canceled)

Applicant: Short, et al.

Serial No.: 10/601,319 Filed

Page : 7 of 21

: June 20, 2003

Attorney's Docket No.: 564462001824 D1370-14US

PATENT

Claim 6 (original): The method of claim 3, further comprising isolating the expressed phytase.

Claims 7 to 10 (canceled)

Claim 11 (currently amended): The method of claim 1-or claim 2, wherein the yeast cell is a Saccharomyces sp., a Schwanniomyces sp., a Pichia sp. yeast cell, a Hansenula sp. yeast cell, a Candida yeast cell or a Torulopsis sp. yeast cell.

Claim 12 (original): The method of claim 11, wherein the yeast cell is a Saccharomyces cerevisiae, a Schizosaccharomyces pombe, a Schwanniomyces occidentalis, a Pichia pastoris or a Hansenula polymorpha.

Claims 13 to 19 (canceled)

Claim 20 (currently amended): The method of claim 1-or-elaim-2, wherein the nucleic acid is contained in further comprises a cloning vehicle.

Claim 21 (currently amended): The method of claim 1 [[20]], wherein the <u>nucleic acid is</u> contained in cloning vehicle comprises an expression cassette, a vector, a plasmid, a phage, a phagemid, a cosmid, a fosmid, a bacteriophage or an artificial chromosome.

Claim 22 (currently amended): The method of claim 1-or claim 2, wherein the polypeptide further comprises a signal peptide and the polypeptide is secreted by the cell.

Claim 23 to 49 (canceled)

Claim 50 (currently amended): The method of claim 1 [[2]], wherein the nucleic acid isolated from an E. coli bacteria is modified to have the [[has a]] sequence of as set forth in SEQ

7

sd-341064

Serial No.: 10/601,319

Filed: June 20, 2003

PATENT

Page : 8 of 21

ID NO:1 or SEQ ID NO:9, or is modified to encode wherein the polypeptide having the [[has an]] amino acid sequence of as set forth in SEQ ID NO:2 or SEQ ID NO:10.

Claim 51 (currently amended): A method for making a recombinant phytase comprising: (a) providing a phytase-encoding nucleic acid operatively linked to a promoter, wherein the nucleic acid comprises a sequence <u>isolated</u> initially derived from <u>an E. coli</u> [[a]] bacterium; and (b) expressing the nucleic acid in a yeast under conditions which allow expression of the recombinant phytase in the yeast.

Claim 52 (currently amended): The method of claim 51, wherein the phytase-encoding nucleic acid nucleic acid has the [[a]] sequence of as set forth in SEQ ID NO:9, or encodes the [[an]] amino acid sequence of as set forth in SEQ ID NO:10, [[and]] or has the sequence of initially derived from a bacterium comprises SEQ ID NO:1, or encodes the [[an]] amino acid sequence of as set forth in SEQ ID NO:2.

Claim 53 (currently amended): A method for making a recombinant phytase comprising: (a) providing a phytase-encoding nucleic acid operatively linked to a promoter, wherein phytase comprises a homologous signal sequence or comprises a heterologous signal sequence in place of the homologous signal sequence, and the nucleic acid comprises a sequence <u>isolated</u> initially derived from an *E. coli* [[a]] bacterium; and (b) expressing the nucleic acid in a yeast under conditions which allow expression of the recombinant phytase in the yeast.

Claim 54 (currently amended): The method of claim 53, wherein the homologous signal sequence or the heterologous signal sequence (signal peptide) signal peptide comprises a secretory signal peptide.

Claim 55 (currently amended): A method for making a recombinant phytase comprising: (a) providing a phytase-encoding nucleic acid operatively linked to a promoter, wherein the

Serial No.: 10/601,319 D1370-14US Filed : June 20, 2003 **PATENT**

Page : 9 of 21

nucleic acid encodes a phytase comprising comprises a homologous signal sequence or the nucleic acid encodes a phytase comprising comprises a heterologous signal sequence in place of the homologous signal sequence, or the nucleic acid [[and]] further comprises a sequence encoding an amino acid sequence comprising an N-terminal identification peptide imparting a desired characteristic[[,]]; and the phytase-encoding nucleic acid comprises a sequence isolated initially derived from an E. coli [[a]] bacterium; and (b) expressing the nucleic acid in a yeast under conditions which allow expression of the recombinant phytase in the yeast.

Claim 56 (currently amended): The method of claim 55, wherein the homologous signal sequence or the heterologous signal sequence (signal peptide) signal peptide comprises a secretory signal peptide.

Claim 57 (currently amended): The method of claim 1-or claim-2, wherein the nucleic acid wherein the nucleic acid is contained in a vector.

Claim 58 (previously presented): The method of claim 57, wherein the vector comprises at least a portion of a nucleotide sequence taken from a cloning vector, an expression vector, a bacterial vector, a plasmid, a viral particle, a phage, chromosomal DNA, nonchromosomal DNA, synthetic DNA, a vaccinia vector, an adenovirus vector, a fowl pox virus, a pseudorabies vector or a combination of nucleotide sequences thereof.

Claim 59 (previously presented): The method of claim 51, wherein the promoter is a constitutive yeast promoter or an inducible yeast promoter.

Claim 60 (currently amended): The method of claim 59, wherein the constitutive yeast promoter is an comprises ADH promoter or a LEU2 promoter or the inducible yeast promoter comprises a GAL promoter.

9 sd-341064

Serial No.: 10/601,319 D1370-14US Filed: June 20, 2003 PATENT

Page : 10 of 21

Claim 61 (previously presented): The method of claim 51, wherein the yeast cell is a Saccharomyces cerevisiae, a Schizosaccharomyces pombe, a Schwanniomyces occidentalis, a Pichia pastoris or a Hansenula polymorpha.

Claim 62 (previously presented): The method of claim 53, wherein the yeast cell is a Saccharomyces cerevisiae, a Schizosaccharomyces pombe, a Schwanniomyces occidentalis, a Pichia pastoris or a Hansenula polymorpha.

Claim 63 (new): The method of claim 51, wherein the phytase-encoding nucleic acid isolated from an *E. coli* bacteria is modified to have the sequence of SEQ ID NO:9, or is modified to encode the polypeptide having the amino acid sequence of SEQ ID NO:10.

Claim 64 (new): The method of claim 55, wherein the phytase-encoding nucleic acid isolated from an *E. coli* bacteria is modified to have the sequence of SEQ ID NO:9, or is modified to encode the polypeptide having the amino acid sequence of SEQ ID NO:10.

Claim 65 (new): The method of claim 55, wherein the phytase-encoding nucleic acid has the sequence of SEQ ID NO:9, or encodes the amino acid sequence of SEQ ID NO:10, or has the sequence of SEQ ID NO:1, or encodes the amino acid sequence of SEQ ID NO:2.

Claim 66 (new): A method for making a recombinant phytase comprising:

- (a) providing a nucleic acid comprising:
- (i) a phytase-encoding nucleic acid isolated from an *E. coli* bacterium and operatively linked to a promoter,

wherein the phytase-encoding nucleic acid encodes a phytase comprising

- (A) a homologous signal sequence, (B) a phytase lacking a homologous signal sequence,
- (C) a heterologous signal sequence in place of the homologous signal sequence, or,
- (D) the phytase-encoding nucleic acid of (A), (B), or (C), further comprising a sequence encoding

Serial No.: 10/601,319 D1370-14US
Filed: June 20, 2003 PATENT

Page : 11 of 21

a coding and/or non-coding sequence or a sequence encoding an amino acid sequence comprising an N-terminal identification peptide imparting a desired characteristic;

(ii) a sequence fully complementary to (i); and

(b) expressing the nucleic acid of (a) in a yeast under conditions which allow its expression in the yeast.

Claim 67 (new): The method of claim 66, wherein the phytase-encoding nucleic acid isolated from an *E. coli* bacteria is modified to have the sequence of SEQ ID NO:9, or is modified to encode the polypeptide having the amino acid sequence of SEQ ID NO:10.

Claim 68 (new): The method of claim 66, wherein the phytase-encoding nucleic acid has the sequence of SEQ ID NO:9, or encodes the amino acid sequence of SEQ ID NO:10, or has the sequence of SEQ ID NO:1, or encodes the amino acid sequence of SEQ ID NO:2.